

AMINO ACID SEQUENCES OF RABBIT SKELETAL β - AND CARDIAC TROPOMYOSINS

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1. Introduction

Tropomyosin is an essential component of the actin-linked calcium regulatory systems in vertebrate skeletal and cardiac muscles. In skeletal muscle there are two major forms of the protein chain, designated α and β , which can be separated by SDS-polyacrylamide gel electrophoresis [1–3] or by ion-exchange chromatography on CM-cellulose in urea buffers [3,4]. The ratio of the α - and β -tropomyosins appears to be related to muscle type with fast white muscles having a higher proportion of the α component (α : β ratio of ~ 3 or $4 : 1$). In slow red and smooth muscles this ratio is close to 1 [3,5,6]. Recent evidence indicates that α -tropomyosin in skeletal muscles is restricted to type II cells [7]. Although it has not yet been established that β -tropomyosin is found only in type I cells, the results clearly imply that the varying ratios are a reflection of the relative proportions of the two classes of cells in various muscle types. In cardiac tissue, the β component appears to be absent in small and fast-beating hearts and present at low levels (15–20% of the total) in larger mammals [8]. In addition rabbit cardiac tropomyosin is similar to pure skeletal α -tropomyosin in terms of its amino acid composition and thiol content, behavior on SDS-polyacrylamide gels, immunological properties and peptide mapping [3,5,9].

The amino acid sequence analysis of rabbit skeletal α -tropomyosin [10–12] and its interpretation by several workers has permitted rational explanations for the stabilization of its coiled-coil structure [10,13–16], for its head-to-tail aggregation as long filaments [15,17,18], for its interaction with 7 actin monomers along each of the two strands of F-actin

[10,16,19,20] and to a suggestion for the site of binding of troponin-T [21]. Here we extend these analyses to both skeletal β -tropomyosin and to cardiac tropomyosin. Our results demonstrate that β -tropomyosin has the same number of residues (284) as α -tropomyosin and therefore does not differ significantly in molecular weight. Thirty-nine amino acid differences are observed in the two sequences. Cardiac tropomyosin is found to be identical in sequence to the skeletal α component.

2. Experimental

Rabbit skeletal β -tropomyosin and rabbit cardiac tropomyosin were prepared from frozen tissue (Pel-Freez Biologicals, Arkansas) by the methods in [12,22]. Each of the two proteins was subjected to chemical cleavage at methionine residues with cyanogen bromide and the resulting peptide mixture fractionated by gel filtration on Sephadex G-75. The smaller peptides were further purified by ion-exchange chromatography on Chromobeads type P and/or high-voltage paper electrophoresis. The two large fragments were separated by ion-exchange chromatography on QAE-Sephadex and each was then further digested with trypsin after citraconylation or with *Armillaria mellea* protease. These approaches and methods have all been described or referenced in [11,12]. Where peptides had the same amino acid compositions, electrophoretic mobilities and NH_2 -terminal analyses as those present in the sequence of α -tropomyosin, they were assumed to be identical. In cases where a difference was indicated by these criteria, the peptides were sequenced either by the automatic Edman

degradation procedure or by the manual dansyl-Edman method. Because of the high similarity or identity of the sequences with α -tropomyosin, peptides were aligned by homology. However in many cases overlap peptides were isolated from the two types of digestions. Some methionine overlap peptides were also isolated by the diagonal method [23] after tryptic digestion of the intact protein.

3. Results and discussion

The complete amino acid sequences of rabbit skeletal β - and cardiac tropomyosins are shown in fig.1. The cardiac sequence was found to be identical to that of skeletal α -tropomyosin and no sequence evidence for heterogeneity was observed during the course of this work. These conclusions are consistent with [3,5,9] that the cardiac and skeletal α forms of the protein have very similar, if not identical, amino acid compositions, peptide maps, polyacrylamide electrophoretic gel mobilities and immunological behavior. The present evidence does not rule out the possibility that other, minor, forms may be present but these could represent only a very small percentage

of the total. This conclusion, of course, applies only to the rabbit cardiac tropomyosin. There is clear evidence that in certain other larger species the cardiac tropomyosins are mixtures of α and β forms [8].

The sequence analysis of skeletal β -tropomyosin shows that its polypeptide chain length (284 residues) is the same as that of α -tropomyosin, with no insertions or deletions. Thus the separation of these two chains on SDS-polyacrylamide electrophoretic gels [1-3] must be due to factors other than significant differences in their molecular weights. Of the total of 39 single amino acid differences between the 2 chains, essentially all involve chemically similar side chains. Two, however, lead to charge changes. Thus serine-229 and histidine-276 are replaced by glutamic acid and asparagine, respectively, in β -tropomyosin. It is presumably these substitutions, giving the β chain a higher net negative charge at most pH values, which permit the separation of the two chains on CM-cellulose in 8 M urea [3].

Analysis of the amino acid sequence of α -tropomyosin has demonstrated the presence of several periodic features [10-16,20]. The most obvious of these features, the repeating pattern of non-polar and polar residues with the 'inner' hydrophobic

C Tm	AcMet-Asp-Ala-Ile-Lys-Lys-Met-Gln-Met-Leu-Lys-Leu-Asp-Lys-Glu-Ala-Leu-Asp-Arg-Ala-Glu-Gln-Ala-Glu-Ala-Asp-Lys-Ile-Ala-Ala-Glu-Asp-Arg-Ser-	5	10	15	20	25	30	35
S β -Tm	-	-	-	-	-	-	-	-
C Tm	-Lys-Gln-Leu-Glu-Asp-Glu-Leu-Val-Ser-Leu-Gln-Lys-Lys-Leu-Lys-Gly-Thr-Glu-Asp-Glu-Lys-Tyr-Ser-Glu-Ala-Leu-Lys-Asp-Ala-Gln-Glu-Lys-Leu-Glu-	40	45	50	55	60	65	70
S β -Tm	-	Glu	Gln	Gln	Val	Glu	Ser	Val
C Tm	-Leu-Ala-Glu-Lys-Lys-Ala-Thr-Asp-Ala-Glu-Ala-Asp-Val-Ala-Ser-Leu-Asn-Arg-Arg-Ile-Gln-Leu-Val-Glu-Glu-Glu-Leu-Asp-Arg-Ala-Gln-Glu-Arg-Leu-Ala-Thr-	75	80	85	90	95	100	105
S β -Tm	-	Gln	-	-	-	-	-	-
C Tm	-Ala-Leu-Gln-Lys-Leu-Glu-Glu-Ala-Glu-Lys-Ala-Ala-Asp-Glu-Ser-Glu-Arg-Gly-Met-Lys-Val-Ile-Glu-Ser-Arg-Ala-Gln-Lys-Asp-Glu-Glu-Lys-Met-Glu-Ile-Gln-	110	115	120	125	130	135	140
S β -Tm	-	-	-	-	-	Asn	Met	-
C Tm	-Glu-Ile-Gln-Leu-Lys-Glu-Ala-Lys-His-Ile-Ala-Glu-Asp-Ala-Asp-Arg-Lys-Tyr-Glu-Glu-Val-Ala-Arg-Lys-Leu-Val-Ile-Ile-Glu-Ser-Asp-Leu-Glu-Arg-Ala-Glu-	145	150	155	160	165	170	175
S β -Tm	-	Met	-	Ser	-	-	Leu	Gly
C Tm	-Glu-Arg-Ala-Glu-Leu-Ser-Glu-Gly-Lys-Cys-Gly-Ala-Glu-Leu-Glu-Glu-Glu-Leu-Lys-Thr-Val-Thr-Asn-Asn-Leu-Lys-Ser-Leu-Glu-Ala-Gln-Ala-Glu-Lys-Tyr-Ser-Gln-	185	190	195	200	205	210	215
S β -Tm	-	Val-Ala	Ser	Gly-Asp	Ile	-	Asp	-
C Tm	-Lys-Glu-Asp-Lys-Tyr-Glu-Glu-Glu-Ile-Lys-Val-Leu-Ser-Asp-Lys-Leu-Lys-Glu-Ala-Glu-Thr-Arg-Ala-Glu-Phe-Ala-Glu-Arg-Ser-Val-Thr-Lys-Leu-Glu-Lys-Ser-	220	225	230	235	240	245	250
S β -Tm	-	-	Leu	-	-	-	Ala	-
C Tm	-Ile-Asp-Asp-Leu-Glu-Asp-Glu-Leu-Tyr-Ala-Gln-Lys-Leu-Lys-Tyr-Lys-Ala-Ile-Ser-Glu-Glu-Leu-Asp-His-Ala-Leu-Asn-Asp-Met-Thr-Ser-Ile	255	260	265	270	275	280	
S β -Tm	-	Val	Met	-	-	Asn	Ile	Leu

Fig.1. Amino acid sequences of rabbit cardiac tropomyosin (C Tm) and rabbit skeletal β -tropomyosin (S β -Tm). The amino acid sequence of C Tm is identical to that of rabbit skeletal α -tropomyosin in [11,12]. The β -Tm sequence is the same at all positions indicated by (—). Only those 39 residue positions which are different are shown.

residues occurring at average intervals of 3.5 residues, is associated with the stabilization of the coiled-coil structure of the molecule. In addition, there exists a 19.7 residue periodicity, repeated 14-fold, which has been interpreted as 2 sets of 7 alternating binding sites involved in the interaction with the 2-strands of actin monomers in the grooves of the F-actin structure. Neither of these 2 types of periodicity is significantly affected by the amino acid substitutions in the β chain as compared with the α .

Of the 39 replacements in the complete β chain, nearly twice as many, 25, occur in the COOH-terminal half of the molecule as in the NH₂-terminal half, 14. Since the troponin-T binding site on tropomyosin may extend over an extended region of the COOH-terminal half of the molecule, this observation may be relevant to the recent demonstration in our laboratory that β -tropomyosin binds less tightly to an immobilized troponin-T fragment than does α -tropomyosin [24]. However this correlation cannot be made more precise until the binding site for troponin tropomyosin has been more definitively established.

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